Evaluation of Chromosome Aberration Effects of Chemicals on Mouse Germ Cells

by W. M. Generoso*

Chromosomal aberrations induced by chemicals represent a class of genetic damage that causes concern as a hazard to the human population. It is well known that many human genetic defects result from chromosomal anomalies of one type or another. However, for two main reasons it has not been easy to evaluate this chemical hazard: first, unlike carcinogenic or teratogenic effects, no agent has yet been implicated as mutagenic to the human population, and many important experiments cannot be done directly on humans; second, the amount of information on the basic nature of chemical effects on mammalian germ cells is still very small. Although there has been a dramatic increase in the number of papers published on mammalian mutagenesis in the past few years, there is still much basic information we need.

So far, evaluation of chromosomal aberrations as genetic hazards of chemicals has been made primarily from dominant-lethal tests in mice or rats and from cytogenetic studies of somatic and germ cells of certain mammals. These two systems, although very useful, do not measure transmissible genetic effects and very little is known about what they mean in terms of hazards. Obviously, the most important mutagenic effects are the permanent transmissible ones. Thus, it is highly desirable from a practical standpoint to have a good understanding of the relationship between all endpoints used to measure chromosomal aberration damage.

Chromosomal aberrations fall into two major classifications: those that result from chromosome breakage and those that arise through chromosomal nondisjunction. When induced in germ cells, either of these events can lead to dominant-lethality or aneuploidy among some of the progeny. In addition to these fates, chromosome breakage can result in progeny with chromosomal rearrangements, primarily reciprocal translocations. This report discusses chromosomal aberrations as chemical effects in mouse germ cells, with emphasis on the types of aberrations chemicals can induce, methods for detecting them, sensitivity of various germ-cell stages, and dose effects of chemicals in the induction of chromosomal aberrations.

Chromosomal Aberrations in Male Germ Cells

Most of the work published on genetic effects of chemicals in mouse germ cells has been on males. Chromosomal aberration effects of chemicals in males have been studied by the use of dominant-lethals, heritable translocation, sex-chromosome loss, and testi-

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cular cytogenetic procedures. The use of the dominant-lethal procedure has been quite extensive and a complete review of this subject will not be made here [more information on dominant-lethal studies is given by Epstein (*1*), Green and Springer (*2*), Salsburg (*3*) and Maxwell (*4*)]. A considerable amount of information on the cytogenetic effects of mutagenic chemicals on mouse spermatogonia has become available in the last two years. This subject, too, will not be touched on, it is discussed thoroughly by Brewen and Preston (*5*). The following discussion includes principally our own work on male mice.

Dominant-Lethal Mutations

Dominant-lethal mutations are genetic changes induced in parental germ cells which lead to death among some of the first-generation embryos. In the mouse, dominant-lethal mutations are generally believed to be mainly chromosome breaks, and embryonic death results from the elimination of affected chromosomes through the breakage–fusion–bridge cycle. The dominant-lethal test is useful for testing the mutagenicity of chemicals. It is a cheap, simple, and quick procedure. Its main limitations are that it screens mainly for chromosome breakage and its sensitivity is capable of screening only highly mutagenic chemicals.

General Procedure for Dominant-Lethal Test in Males as Used in our Laboratory. Twenty adult (10- to 12-week-old) male mice, each from two unrelated strains, are injected intraperitoneally with the test compound at approximately LD_{10} level. Lower doses are used if the mating ability of treated males is impaired. Corresponding control mice are injected with the same volume of carrier solution. Immediately after treatment each male is paired with two suitable females. Every morning, females are examined for presence of vaginal plugs (indication of mating) and each female that copulated is removed and replaced by a virgin female. This routine is carried on for a period of 48 days, which is sufficient to cover the entire spermatogenic cycle. All mated females are killed for uterine analysis at 12 to 15 days after observation of the vaginal plug.

Mutagenicity of the test compound is decided by a combination of the following criteria: (1) increase in the frequency of dead implantations, (2) reduction in the average number of living embryos, (3) reduction in the average number of implantations, and (4) reduction in the frequency of fertile matings. Generally, the first and second and, in some cases (when induction rate is high), also the third criteria are expressed together. The fourth criterion is expressed only when dominant-lethal induction approaches 100%. Apparent sterility of some treated males may be due to dominant-lethality or to physiologic reasons such as the inability of treated males to mate during the posttreatment sick phase; thus the value of checking for vaginal plugs. For analysis, data are pooled into successive 2-day intervals.

It should be noted that the sensitivity of this procedure depends very heavily on the reproductive nature of the females. It is, therefore, important to use a strain of females possessing the following qualities: (1) large litter size; (2) low frequency of dead implantations; (3) high proportion of matings, as indicated by vaginal plugs, during the receptive stage of the estrous cycle; and (4) uniformity among females.

Spermatogenic Stage Differences in Sensitivity to Dominant-Lethal Induction. A great number of chemicals had already been studied for induction of dominant-lethal mutations in male mice. Many of these chemicals, primarily alkylating agents and those that are transformed into an alkylating form, are highly effective in inducing dominant-lethal mutation at specific stages in spermatogenesis. To illustrate stage differences, only seven of the definite dominant-lethal inducers will be considered (*6–14*).

Chemicals differ dramatically in the stages at which dominant-lethals can be induced. For instance, in the meiotic and postmeiotic stages, isopropyl methanesulfonate (IMS)

and triethylenemelamine (TEM) induce dominant lethals in spermatozoa, spermatids, and spermatocytes; ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), and *n*-propyl methanesulfonate (PMS) only in spermatozoa and spermatids; Mitomycin C, in spermatids and spermatocytes; and 1,4-di(methanesulfonoxy)butane (Myleran) in spermatozoa and spermatocytes. Within one stage there can be a marked difference in sensitivity. For EMS, early spermatozoa and late spermatids are more sensitive than late spermatozoa and early spermatids, respectively. Such differences within a stage are also true for other chemicals, although in varying degrees. From the practical standpoint of screening mutagenic chemicals, the marked sensitivity differences that may exist within one stage should be taken into consideration. Since females vary in the stage of the estrous period when they are put with the test males, fertilization usually occurs at random. If females are not checked for plugs and the mating period is on a weekly basis, a dominant-lethal effect may be missed by chance. Obviously this consideration is more important when the dominant-lethal effect is small and when low numbers of animals are used. Thus the efficiency of the dominant lethal test can be considerably increased by checking for plugs and analyzing the matings at closer intervals.

Although relatively fewer data are available on the sensitivity of spermatogonia to dominant-lethal induction with chemicals, it is already obvious that even if dominant-lethals are induced, the level is very low and difficult to measure. In fact, there is no convincing evidence available which shows that chemicals can induce a detectable increase in dominant-lethal mutations in spermatogonia. Our results with a single TEM dose of 4.0 g/kg indicates that TEM does not induce a detectable increase in dominant-lethal mutations in spermatogonia (*12*), while only 0.2 mg/kg is required to induce approximately 50% dominant-lethals in the most sensitive postspermatogonial stage. Contrary to TEM, a positive dominant-lethal effect has been claimed for cyclophosplasmide (*15*) and Mitomycin C (*16*). However, the data for these two compounds are not extensive enough, hence there is still doubt on their effectiveness in inducing dominant-lethals in spermatogonia. Certain chemicals, such as TEM, IMS, Myleran, and Mitomycin C, are cytotoxic to spermatogonia, resulting in temporary sterility of treated males.

Strain difference is another factor which must be considered in testing chemicals for induction of chromosomal aberrations in male mice. A clear-cut strain difference was found for EMS induction of dominant-lethal mutations (*6*). At a dose of 300 mg/kg, EMS-induced dominant-lethal mutations which resulted in complete sterility in all treated early spermatozoa (matings that occurred 6½ to 7½ days after treatment) in the (101 X C3H)F_1 males, while in the T-stock males the majority of mated females were fertile, with an average of more than two living embryos per pregnant female. This strain difference also exists at the dose of 200 mg/kg. Such strain difference is obviously a very important consideration, especially when the mutagenic effect of the test compound is low.

Heritable Reciprocal Translocations

One of the consequences of chromosome breakage induced in the postmeiotic stages is the production of symmetrical reciprocal translocations which can be passed to some of the first generation progeny. Such progeny are thus heterozygotes for the translocation. A number of chemicals that are effective dominant-lethal inducers have also been found to be effective in inducing heritable reciprocal translocations in the same germ cell stages. This correlation strengthens the belief that induced dominant-lethals are primarily breaks. Screening for translocation heterozygosity in the F_1 progeny can be done either by cytological analysis or by fertility testing and is more readily accomplished in males than in females. Cytological screening of F_1 male progeny of irradiated males was carried out by

Leonard and Deknudt (*17*) by using the air drying method of Evans, Breckon, and Ford (*18*) and analyzing 100 diakinesis-metaphase I spermatocytes per mouse. This method of screening for male translocation heterozygotes has the advantage of requiring very little animal space to test each F_1 male. On the other hand, it is obviously relatively less suitable for large-scale screening than the fertility test.

Male translocation heterozygotes may be either partially or completely sterile. In the course of our study on dose effects of EMS (*19*) (see below), we accumulated information on the fertility of male translocation heterozygotes. A total of 148 sterile and partially sterile F_1 male progeny were recovered. Of these, 98 were partially sterile and 50 were completely sterile. For 96 of the 98 partially sterile males, translocation heterozygosity was confirmed by cytological examination of diakinesis-metaphase I, spermatocytes, and transmission of partial sterility to the next generation. The other two confirmed partially sterile male, died before tests could be completed. Comparison between the fertility of 98 EMS-induced partially sterile male translocations with a much larger number of normal males revealed that the fertility of partially sterile males is only 43–44% that of normal males. Furthermore, data on sacrificed females show that reduction in the number of living embryos is attributable mainly to an accompanying increase in dead implantations and a small but significant proportion of embryonic loss that occurred in early cleavage stages. There is no doubt that EMS-induced partial sterility among F_1 male progeny is associated with induced reciprocal translocations. Cytogenetic analysis of partially sterile males, transmission of partial sterility, and the study of Cattanach. Pollard, and Isaacson (*20*) on EMS-induced partial sterility in males support this conclusion. Furthermore, partial sterility in F_1 male progeny induced by radiation and other chemicals has almost always been associated with induced reciprocal translocation (*21*). These observations clearly establish that in males, partial sterility alone can be taken as unequivocal indication of a reciprocal translocation.

As indicated already, of the EMS-induced sterile and partially sterile F_1 male progeny, about one-third are sterile and two-thirds are partially sterile. In a much lower number of F_1 males tested, Cattanach, Pollard, and Isaacson (*20*) obtained about equal numbers of the two classes. There is now clear evidence which suggests that at least the great majority of induced sterility among F_1 males, like partial sterility, is attributable to induced chromosomal rearrangement. Cytological evidence was discussed by Cattanach, Pollard, and Isaacson, (*20*), and more recently provided by Cacheiro, Russell, and Swartout (*22*).

General Procedure Used in Our Laboratory for Screening Translocation Heterozygotes. The conventional procedure for detecting translocation heterozygotes is to test F_1 male progeny of treated parents for sterility or partial sterility by mating each one to three or more different females. Each female is opened at midpregnancy, and the living and dead implants are counted. This procedure for detecting translocation males is obviously expensive and requires a good deal of animal handling and record keeping. The procedure we are currently using is still under development but is already more suitable for wide-scale screening and experimentation.

This procedure is the result of our extensive study on the fertility of EMS-induced partially sterile translocations (*19*). The procedure as it stands now is as follows. The key to this screening procedure is the exceptional fertility of (SEC X C57BL)F_1 females. Each F_1 male to be tested is caged with an (SEC X C57BL) F_1 female. The females are used for the first time when they are 10 to 78 weeks old. Breeding pens are checked for newly born mice when they are expected, i.e., pens are examined daily during weekdays beginning 18 days after pairing and 18 days after appearance of a litter. Young are discarded immediately

after they are scored. For each male, if the size of the first litter is 10 or more, the male is declared fertile and discarded immediately after scoring the litter. If the first litter is less than 10, a second litter is scored. If the second litter is 10 or more, the male is declared fertile and discarded, otherwise the male is a suspect and is tested further by mating him to three virgin females which are killed during pregnancy. In either case, another male is placed with the female one week after the litter is born and the same procedure is followed until the last male is added no later than the tenth litter. The lapse of one week is required so that parentage of litters will not be confused. From our study on the fertility of partially sterile translocation heterozygotes, the probability that a partially sterile male will sire a litter of size 10 or more is estimated as 0.02.

Analysis of the fertility data of the (SEC X C57BL)F_1 females mated with fully fertile males revealed that, if litter size of 10 or more in his first or second liter sired by a male is used as the indicator of full fertility and each female is allowed to produce 12 consecutive litters, an average of 5.27 males from a sample free of translocations may be tested per female. Out of these, the number of test males per female that can be declared fully fertile on the basis of one or two litters sired is estimated as 4.20. Thus it is now possible to test several F_1 males per female instead of killing three or more females per F_1 male as in the old procedure. The new procedure is now routinely used in our laboratory for screening male translocation heterozygotes in chemical and radiation experiments.

Dose Effects of Chemicals in Induction of Dominant-Lethal Mutations and Heritable Translocations in Males.

Dose is a very important consideration in the evaluation of mutagenicity of chemicals. Yet, information on the dose effect of chemicals in the induction of genetic damage to mammalian germ cells is meager. We are studying this problem in depth and results of our early studies are now available. Generoso et al. (*19*) studied the effect of EMS dose on the induction of dominant-lethal mutations and transmissible reciprocal translocations in the same postmeiotic germ cell stage and compared the shapes of the dose-effect curves and efficiency for measuring chromosome breakage between the two endpoints. Matter and Generoso (*23*) studied the effects of TEM dose on the induction of dominant-lethal mutations and compared the shape of the TEM dose-effect curve with that of EMS.

The EMS dose-effect study was performed with doses ranging from 50 to 300 mg/kg. It was found that for both dominant-lethals and translocations the effectiveness of EMS in inducing chromosome breakage is proportionately much lower at low doses. The dominant-lethal dose-response curve is clearly not linear—it is markedly concave upward. Similarly, the translocation dose-response curve showed that there is a more rapid increase in the number of translocations with dose than would be expected on the basis of dose-square kinetics. One important aspect, from a practical standpoint, of this dose-response study is the finding that, whereas induced dominant-lethal mutations are convincingly detected at 150 mg/kg and above, a significant increase in induced translocations was already detectable at the 50 mg/kg dose. It is likely that dominant-lethal mutations had also been induced at doses lower than 150 mg/kg but were not detected, owing to the relative insensitivity of the dominant-lethal procedure. Our early results with TEM also indicate a similar relationship between the two endpoints. In addition to the higher sensitivity of this translocation procedure, translocations are a much more reliable endpoint in terms of human hazards than dominant-lethal mutations because, unlike the latter, they represent transmissible genetic damage. Thus, for the detection of low levels of chromosome breakage, translocations are a more reliable endpoint than dominant-lethal mutations.

From the dominant-lethal dose-response studies, two interesting differences, which have basic as well as practical significance,

were found between EMS and TEM. First, a marked difference exists in the shape of the dose-response curve between EMS and TEM. Whereas the EMS dose-effect curve departs markedly from linearity, the TEM dose-effect curve (either for spermatozoa or spermatids) approximates linearity. At present, there is very little information available to explain this large difference. Yet this is a very important fundamental problem since it touches on the basic mechanisms involved in the production of chromosome breaks by chemicals.

Another interesting difference between EMS and TEM that has importance from the practical point of view, lies in their relative effectiveness in inducing chromosome breakage at low dose levels. The ratio of genetically effective dose (as measured by induced dominant-lethals) to lethal dose for TEM is 1/100, while for EMS it is only 1/3.5. In fact, our early results with reciprocal translocation already indicate that TEM is mutagenic at doses as low as 1/200, or less, of the lethal dose. Thus it is clear that TEM, in contrast to EMS, is mutagenic far below the toxic level. It is interesting to point out that, in two different strains of mice, Jurand (*24*) found that relatively high doses of TEM (more than 50 times that of the lowest dose which induces detectable chromosome breakage) given to pregnant females were needed before any retardation of fetal development or teratological effects could be detected. The finding that TEM is mutagenic at extremely low doses strengthens the belief that chemicals in the human environment constitute a potential genetic hazard and that chromosome breakage and its consequences are important components of this hazard.

Paternal Sex-Chromosome Loss and Nondisjunction

The discovery in mice that XO sex-chromosome constitution is viable fertile female (*25, 26*) and XXY is viable male (*27*) has spearheaded the use by Russell and Russell of various X-linked markers in the measurement of radiation-induced sex-chromosome anomalies (*28*). The general procedure was first outlined by L. B. Russell (*29*). With appropriate markers both XO female and XXY male progeny can be phenotypically detected. The sex chromosome constitution can be verified by either genetic or cytological tests.

With chemicals, the procedure was first used by Cattanach (*30*), who found that TEM is effective in inducing sex-chromosome loss following treatment of postmeiotic germ cells. Sex chromosome loss induced in postmeiotic germ cells is presumably a consequence of chromosome breakage. For TEM as well as x-rays, this is supported by the fact that dominant-lethals and translocations are readily induced in the postmeiotic stage. Moutschen (*31*) also used the method to study induction of sex-chromosome loss and nondisjunction at all stages in spermatogenesis with MMS. However, it was a small-scale and incomplete study and the results are not conclusive.

We are presently using this method for detecting sex-chromosome anomalies in males, primarily in our study of the overall induction of chromosomal aberrations in the mouse spermatogonia, specifically sex-chromosome nondisjunction and loss.

Chromosomal Aberrations in Female Mice

It is clear, from direct evidence, that certain chemicals are highly effective in inducing chromosome breakage at specific stages in spermatogenesis. In females, on the other hand, although it appears certain that some chemicals are also effective in inducing chromosomal aberrations, evidence available in the literature is all of indirect nature.

Induction of Dominant-Lethal Mutations in Mouse Oocytes

A number of chemicals that are known to be effective dominant-lethal inducers in male postmeiotic germ cells have been studied for induction of dominant-lethal mutations in adult female mice. Some of these chemicals are TEM (*32, 33*), EMS (*34*), MMS (*34*), IMS (*33*), Myleran (*33*), and

Trenimon [2, 3, 5-tris(ethyleneimino)-*p*-benzoquinone] (*35*). At the time of treatment, all germ cells, with the exception of those that will be ovaluated within a few hours, are at the primary oocyte stage—specifically at diffused diplotene. In all cases, presumed dominant-lethal effects in treated females were similar in manifestation to those when males received the treatment, i.e., increased pre- and postimplantation embryonic death. However, since the females themselves received chemical treatment, the distinction between genetic and toxic effects as the cause of embryonic loss is very difficult to make. There is indirect evidence which points to the genetic nature of the embryonic mortality among chemically treated females. First, these chemicals are effective inducers of dominant-lethal mutations in male mice. Second, it was found that preimplantation loss of embryos following prefertilization treatment of female mice with EMS was associated with an increase in the frequency of subnuclei among embryos in early cleavage stages (*34*). Third, IMS-induced aborted meiosis among oocytes in the advanced stages of follicular development leading to cell death (*33*). And fourth, a significant increase in the frequency of chromosome aberrations was cytologically detected among two-cell embryos from female mice treated with trenimon shortly before induced ovulation (*36*). These findings, although by no means unequivocal evidence, strongly suggest a genetic basis for the embryonic lethality induced by these chemicals. Additional genetic evidence for IMS is described in the last paragraph of this report.

Large strain differences exist in the dominant-lethal response of female mice to mutagenic chemicals (*6, 33, 37*). Chemicals differ in their effectiveness and in the manifestation of dominant-lethal effects, but in all cases the primary effect is the production of postimplantation embryonic death and, with certain chemicals, also preimplantation losses at high doses. It is clear that a given strain may be more sensitive to one chemical than another, and that the order of sensitivity to chemicals may differ among the strains.

In a recent report (*37*), it was attempted to explain why studies on females, in addition to those on males, should be incorporated in the assessment of the fertility effects of chemicals. It was reasoned that fertility effects of chemicals in females may represent a wide variety of possible health hazards such as mutagenic, cytotoxic, and other not easily identifiable nongerminal undesirable effects. Another reason why it may be necessary to include females in the fertility study in mammals is clearly illustrated by our recent finding (*38*) with the compound hycanthone. Results in males show that hycanthone does not induce any detectable increase in dominant-lethal mutations in two strains of mice, although reduction in fertility due to cytotoxic damages to spermatogonial cells was observed. In females, on the other hand, hycanthone is effective in inducing increases in the incidence of dead implantations. It should be pointed out that whether this response is due to genetic or toxic effects is not known at the present time. A somewhat similar situation seems to be true for IMS in which there are indications that with certain strains of mice females may be more sensitive to induction of dominant lethals than males (*7, 33*).

Cytogenetic Analysis of Chemical Damage to Mouse Oocytes

Chromosomal aberration effects of chemicals in mouse oocytes have been studied either directly on the oocytes that are in M–I or M–II or indirectly on early embryonic stages. In both cases, experimental females were given hormonal pretreatment to synchronize estrus. The test chemicals were administered at appropriate periods which correspond to the desired meiotic stages. Jagiello (*39–41*) found that the antibiotics phleomycin and streptonigrin induced various abnormalities that were detectable in M–I and M–II, while no effect was obtained for three mercury compounds. By direct examination of M–II oocytes Röhrborn and Hansmann (*42*) found that the compounds trenimon, cyclophosphamide, and methotrexate are highly effective in inducing struc-

tural and numerical chromosomal aberrations among treated preovulatory oocytes. Similarly, analysis of two-cell embryos from Trenimon-treated oocytes also showed high incidence of structural and numerical aberrations (*36*).

Because of the apparent effectiveness of the chemicals mentioned above in inducing cytologically detectable chromosomal damage, it is important to examine further this approach in female mice. Since such cytogenetic data came from only two laboratories, it seems appropriate to have similar studies conducted by other workers. More importantly, the test may be done without the use of hormonal pretreatment so that only normally developing oocytes are studied, and the possibility of synergism between the hormones and the test chemicals is eliminated. Furthermore, cytogenetic analysis may also be done on oocytes treated not only within a few hours prior to ovulation but also on those that are in other stages of follicular development. Such information can be matched up with data on presumed dominant-lethal mutations.

Induction of Heritable Chromosomal Aberrations in Mouse Oocytes

The results of dominant-lethal and cytogenetic studies in female mice, although suggestive of genetic damage, do not represent transmissible aberrations, and it is not known at present what they mean in terms of hazard. Furthermore, our inability to determine whether the fertility effects of hycanthone in female mice are due to induced genetic damage clearly illustrates the necessity for a reliable genetic assay system for evaluating mutagenic hazards of chemicals in mouse oocytes. Thus it would certainly be highly desirable to determine the relationship of the presumed dominant lethals and cytogenetic damage to heritable chromosome damage. For these reasons, we are currently studying the ability of certain chemicals to induce sex-chromosome loss and heritable reciprocal translocations. The initial experiment is being conducted with IMS. Although this study is not yet complete, it is already obvious that IMS is effective in inducing sex-chromosome loss on mouse oocytes (*43*). It may be noted that this information represents the first clear-cut genetic evidence for chemically induced chromosomal aberration in mouse oocytes.

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REFERENCES

1. Epstein, S. S. Use of the dominant-lethal test to detect genetic activity of environmental chemicals. Environ. Health Perspect. **No. 6**: 23 (1973).
2. Green, S., and Springer, J. A. The dominant-lethal test: Potential limitations for safety evaluation. Environ. Health Perspect. **No. 6**: 37 (1973).
3. Salsburg, D. S. Statistical considerations for dominant-lethal mutagenic trials. Environ. Health Perspect. **No. 6**: 51 (1973)
4. Maxwell, A., and Newell, G. W. Considerations for evaluating the chemical mutagenicity to germinal cells. Environ. Health Perspect. **No. 6**: 47 (1973).
5. Brewen, J. G., and Preston, R. J. Chromosome abbreviations as a measure of mutagenesis: *in vivo* versus *in vitro* and somatic versus germ cell comparisons. Environ. Health Perspect. **No. 6**: 157 (1973).
6. Generoso, W. M., and Russell, W. L. Strain and sex variations in the sensitivity of mice to dominant-lethal induction with ethyl methanesulfonate. Mutation Res. **8**: 589 (1969).
7. Ehling, U. H., Cumming, R. B., and Malling, H. V. Induction of dominant lethal mutations by alkylating agents in male mice. Mutation Res. **5**: 417 (1968).
8. Ehling, U. H. Comparison of radiation- and chemically-induced dominant lethal mutations in male mice. Mutation Res., **11**: 35 (1971).
9. Ehling, U. H., and Malling, H. V. 1,4-Di(methane-sulfonoxy)butane (Myleran) as a mutagenic agent in mice. Genetics, **60**: 174 (1968).
10. Cattanach, B. M., and Edwards, R. G. The effects of triethylenemelamine on the fertility of male mice. Proc. Roy. Soc. Edinburgh, **67**: 54 (1958).

11. Matter, B. E., and Generoso, W. M. Effects of dose on the induction of dominant-lethal mutations with triethylenemelamine (TEM) in male mice. In preparation.
12. Generoso, W. M. Induction of dominant-lethal mutations in the mouse spermatogonia with TEM. Unpublished
13. Ehling, U. H., and Neuhauser, A. Stage-specific induction of mutations by alkylating agents in mice. Paper presented at the 1st Annual Meeting of the European Environmental Mutagen society, Noordwijkerhaut, The Netherlands, May 5–7, 1971.
14. Partington, M., and Bateman, A. J. Dominant-lethal mutations induced in male mice by methyl methanesulfonate. Heredity 19: 191 (1964).
15. Brittinger, D. Die mutagene Wirkung von Endoxan bei der Maus. Humangenetik 3: 156 (1966).
16. Ehling, U. H., and Neuhauser, A. Stage-specific induction of mutations by alkylating agents in mice. Paper presented at the 1st Annual Meeting of the European Environmental Mutagen Society, Noordwijkerhant, The Netherlands, May 5–7, 1971.
17. Leonard, A., and Deknudt, G. H. The sensitivity of various germ-cell stages of the male mouse to radiation induced translocations. Can. J. Genet. Cytol. 10: 495 (1968).
18. Evans, E. P., Breckon, G., and Ford, C. E. An air-drying method for meiotic preparations from mammalian testes. Cytogenetics 3: 289 (1964).
19. Generoso, W. M., Russell, W. L., Huff, W. W., and Stout, S. K. Effect of dose on the induction of chromosome aberrations with ethyl methanesulfonate (EMS) in male mice. Paper presented at 2nd Annual Meeting of the European Environmental Mutagen Society, Zinkovy Castle, Czechoslovakia, May 10–12, 1972; Abstracts p. 21; full paper in preparation.
20. Cattanach, B. M., Pollard, C. E., and Isaacson, J. H. Ethyl methanesulfonate-induced chromosome breakage in the mouse. Mutation Res. 6: 297 (1968).
21. Russell, L. B. Chromosome aberrations in experimental mammals. In A. G. Steinberg and A. G. Bearn, Eds., Progress in Medical Genetics, Vol. 2, Grune and Stratton, New York, 1962, pp. 230–294.
22. Cacheiro, N. L. A., Russell, L. B., and Swartout, M. S. Cytological causes of F_1 male sterility after mutagenic treatment. In preparation.
23. Matter, B. E., and Generoso, W. M. Dose-response studies on the induction of dominant lethal mutations in postspermatogonial stages of the mouse treated with triethylenemelamine (TEM). Paper presented at 2nd Annual Meeting of the European Environmental Mutagen Society Zinkovy Castle, Czechoslovakia, May 10–12, 1972; Abstracts, pp. 40–42; full paper in preparation.
24. Jurand, A. Action of triethylenemelamine (TEM) on early and late stages of mouse embryos. J. Embryol. Exp. Morph. 7: 526 (1959).
25. Russell, W. L., Russell, L. B., and Gower, J. Exceptional inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex-chromosome constitution in female. Proc. Natl. Acad. Sci. U.S., 45: 554 (1959).
26. Welshons, W. J., and Russell, L. B. The Y-chromosome as the bearer of male-determining factors in the mouse. Proc. Natl. Acad. Sci. U.S., 45: 560 (1959).
27. Russell, L. B., and Chu, E. H. Y. An XXY male in the mouse. Proc. Natl. Acad. Sci. U.S., 47: 571 (1961).
28. Russell, L. B. The use of sex-chromosome anomalies for measuring radiation effects in different germ-cell stages of the mouse. Proceedings of IAEA Study Group Meeting in Vienna, Austria, May 8–11, 1967. In Effects of Radiation on Meiotic Systems, pp. 27–41, 1968.
29. Russell, L. B., and Saylors, C. L. The relative sensitivity of various germ-cell stages of the mouse to radiation-induced nondisjunction, chromosome losses and deficiencies. In Repair from Genetic Radiation Damage, F. Sobels, Ed., Pergamon Press, Oxford–London–New York–Paris, 1963, pp. 313–342.
30. Cattanach, B. M. Induction of paternal sex-chromosome losses and deletions and of autosomal gene mutations by the treatment of mouse post-meiotic germ cells with triethylenemelamine. Mutation Res. 4: 73 (1967).
31. Moutschen, J. Mutagenesis with methyl methanesulfonate in mouse. Mutation Res., 8: 581 (1969).
32. Cattanach, B. M. The effect of triethylenemelamine on the fertility of female mice. Int. J. Rad. Biol. 3: 288 (1959).
33. Generoso, W. M., Huff, S. W., and Stout, S. K. Chemically induced dominant-lethal mutations and cell killing in mouse oocytes in the advanced stages of follicular development. Mutation Res. 11: 411 (1971).
34. Generoso, W. M. Chemical induction of dominant lethals in female mice. Genetics 61: 461 (1969).
35. Rohrborn, G. Über einen Geschlechtunterschied in der mutagenen Wirkung von Trenimon bei der Maus. Humangenetik 2: 81 (1966).
36. Rohrborn, G., Kuhn, O., Hansmann, I., and Thor, K. Induced chromosome aberrations in early embryogenesis of mice. Humangenetik, 11: 316 (1971).
37. Generoso, W. M., and Cosgrove, G. E. Total reproductive capacity procedure in female mice. In Chemical Mutagens: Principles and Methods for Their Detection, A. Hollaender, Ed., Vol. 3, Plenum Press, New York–London, in press.

38. Generoso, W. M., Cain, K. T., and De Serres, F. J. Studies on the induction of dominant-lethal mutations with hycanthone in male and female mice. In preparation.
39. Jagiello, G. Streptonigrin: Effect on the first meiotic metaphase of the mouse egg. Science 157: 453 (1967).
40. Jagiello, G. Action of phleomycin on the meiosis of the mouse ovum. Mutation Res. 6: 289 (1968).
41. Jagiello, G., and Lin, J. S. An assessment of the effects of mercury on the meiosis of mouse ova. Mutation Res. 17: 93 (1973).
42. Rohrborn, G., and Hansmann, I. Induced chromosome aberrations in unfertilized oocytes of mice. Humangenetik 13: 184 (1971).
43. Generoso, W. M., Cain, K. T., Huff, S. W., and Rutledge, J. C. Chemical induction of sex-chromosome loss in female mice. Proceedings XIII Int. Congr. of Genetics, Berkeley, California, U.S.A., August 20–29, 1973.